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Metabolic response to green tea extract during rest and moderate-intensity exercise☆

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Abstract

Background: Green tea catechins have been hypothesized to increase energy expenditure and fat oxidation by inhibiting catechol-*O*-methyltransferase (COMT) and thus promoting more sustained adrenergic stimulation. Metabolomics may help to clarify the mechanisms underlying their putative physiological effects.
Objective: The study investigated the effects of 7-day ingestion of green tea extract (GTE) on the plasma metabolite profile at rest and during exercise.
Methods: In a placebo-controlled, double-blind, randomized, parallel study, 27 healthy physically active males consumed either GTE (n=13, 1200 mg catechins, 240 mg caffeine/day) or placebo (n=14, PLA) drinks for 7 days. After consuming a final drink (day 8), they rested for 2 h and then completed 60 min of moderate-intensity cycling exercise (56%±4% VO_2max). Blood samples were collected before and during exercise. Plasma was analyzed using untargeted four-phase metabolite profiling and targeted profiling of catecholamines.
Results: Using the metabolomic approach, we observed that GTE did not enhance adrenergic stimulation (adrenaline and noradrenaline) during rest or exercise. At rest, GTE led to changes in metabolite concentrations related to fat metabolism (3-β-hydroxybutyrate), lipolysis (glycerol) and tricarboxylic acid cycle (TCA) cycle intermediates (citrate) when compared to PLA. GTE during exercise caused reductions in 3-β-hydroxybutyrate concentrations as well as increases in pyruvate, lactate and alanine concentrations when compared to PLA.
Conclusions: GTE supplementation resulted in marked metabolic differences during rest and exercise. Yet these metabolic differences were not related to the adrenergic system, which questions the *in vivo* relevance of the COMT inhibition mechanism of action for GTE.
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Keywords: Green tea; Catechin; Metabolomics; Exercise; Catecholamine; Energy metabolism

1. Introduction

The concept of "challenging homeostasis" has been proposed as a novel approach to define biomarkers for nutritional related health [1]. It is thought that responses to a challenge of homeostasis will affect multiple pathways and provide information other than static homeostatic and disease-relevant end-point measures. These dynamic responses may be appropriate means to assess effects and mechanisms of nutritional interventions that may remain hidden under the large variation within a healthy population. Exercise is a physiological challenge to homeostasis of the human body. Metabolomics based on gas or liquid chromatography–mass spectrometry (GC/MS, LC/MS) is capable of measuring the multifactorial, integrative metabolic responses to an exercise challenge and/or a nutritional intervention or supplement [2–6].

Green tea has been reported to have a number of health-promoting effects, including antiobesity properties [7–10]. These health benefits are generally attributed to its polyphenol content, particularly to catechins that are often enriched in green tea extract (GTE). These are comprised epigallocatechin gallate (EGCG), epicatechin gallate and gallocatechin gallate, among others. EGCG is thought to be the most pharmacologically active of the catechins. GTE also contains caffeine. The antiobesity properties of GTE are attributed to both acute [11] and chronic [7,12] GTE supplementation augmenting energy expenditure (EE) and fat oxidation under resting conditions. However, this has not been consistently reported [13,14]. During moderate-intensity exercise, EE is elevated several times when compared to rest, and absolute rates of lipolysis and fat oxidation are 3–10-fold higher. The administration of GTE could at least in theory have an additive effect on fat metabolism above and beyond what is seen with exercise alone. Indeed, it has been observed that fat metabolism is up-regulated during exercise following both acute [15] and chronic [16] GTE supplementation. So far, differences in the metabolic effects of GTE between rest and exercise have not been investigated in a single study. It is also currently unknown whether the effects of GTE on fat metabolism or any other related metabolic effects are more prominent following acute or chronic GTE supplementation.

It has previously been observed that certain catechins in GTE target specific control points of the sympathetic nervous system [17].

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EGCG has been suggested to directly inhibit catechol-*O*-methyltransferase (COMT), an enzyme that is responsible for degrading catecholamines [noradrenaline (NA) and adrenaline (A)] [18]. Inhibiting the degrading action of COMT would lead to elevated catecholamine concentrations, resulting in greater sympathetic nerve stimulation and thus higher rates of lipolysis. This is thought to elevate fat oxidation and EE at rest and during exercise [10]. The COMT pathway is likely to take effect following acute GTE supplementation. Alternatively, it has been suggested that chronic GTE supplementation may be the lead to an up- or down-regulation of various proteins and enzymes involved in fat metabolism [19,20]. However, there is no convincing evidence to support the acute or chronic mechanisms of GTE *in vivo*.

Based on the mixed study outcomes on fat metabolism following GTE supplementation and the lack of mechanistic evidence *in vivo*, the aim of the present study was to examine the effects of 7 days of GTE supplementation (1200 mg total catechins and 240 mg caffeine/day) on human metabolism at rest and during moderate-intensity exercise (56±%4% VO_2max). These effects were assessed in human plasma using GC–MS- and LC–MS/MS-based four-phase metabolite profiling [21]. This comprehensive metabolite profiling approach provided an unbiased and systemic investigation into the multifactorial metabolic response following GTE supplementation at rest and during exercise. We hypothesized that GTE ingestion for 7 days would induce metabolic changes consistent with increased lipolysis and that these changes could be maintained or enhanced during exercise after a single bolus intake of GTE. In addition, we tested the hypothesis that GTE stimulates the adrenergic system by measuring targeted profiles including various catecholamines.

2. Methods and materials

2.1. Study design

2.1.1. Participants

Twenty-seven healthy physically active male participants were recruited for the purposes of the study. Inclusion criteria were as follows: less than four cups of tea or coffee/day (thus less than ~400 mg caffeine/day), exercise 3–5 times/week, 30–90 min/session. Participants were randomly allocated into either a GTE group [n=13, age 22±5 years, weight 77.6±12.0 kg, body mass index (BMI) 24.3±3.0 kg/m^2] or a placebo (PLA) group (n=14, age 22±8 years, weight 78.8±10.2 kg, BMI 24.7±2.7 kg/m^2). All participants gave written informed consent to participate in the study. The study was approved by the University of Birmingham Ethics Committee.

2.1.2. Preliminary testing

One week prior to the first experimental trial, all participants visited the human performance lab for familiarization, assessment of health and an incremental exercise test using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) to volitional exhaustion (FatMax) [22]. Participants started by cycling at 95 W for 3 min, and work rate was increased by 35 W (W_{inc}) every 3 min (t_{inc}) until they reached exhaustion. The W_{max} was calculated by using the following equation [23]:

$$W_{max} = W_{out} + (t / t_{inc})W_{inc}$$

W_{out} is the power output of the last completed stage (in W), and t is the time (in minutes) spent in the final stage. W_{max} values were used to determine the workload of 50% W_{max} (~55% VO_2max) used in the experimental trials. Respiratory measures of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were assessed using an online gas analysis system (Oxycon Pro, Jaeger). Heart rate (HR) was measured continuously (Polar S625X; Polar Electro Oy, Kempele, Finland). VO_2 was considered to be maximal if two of the following three conditions were met: (a) a leveling off of (VO_2) with further increasing workloads; (b) an HR within 10 beats/min of the age-predicted maximum (220 bpm−age) and (c) a respiratory exchange ratio >1.

2.1.3. Experimental design

The study was designed as a placebo-controlled, double-blind, randomized, parallel study. Subjects in the assigned groups consumed either a GTE drink (1200 mg catechins, 240 mg caffeine) or a PLA drink for 7 days (Fig. 1).

On day 0 (D0), all participants arrived at the Human Performance Lab between 06:00 and 08:00 following a 10-h overnight fast and 24-h controlled diet on D0. Upon arrival, a flexible 20-gauge Teflon catheter (Venflon; Becton Dickinson, Plymouth, United Kingdom) was then inserted into an antecubital vein. A three-way stopcock (PVB Medizintechnik, Kirchseean, Germany) was attached to the catheter to allow repeated blood sampling during the trial. A resting blood sample (5 ml) was taken (t=0 min). Participants then rested for 2 h in a seated position. At the end of the rest period, a second resting blood sample (5 ml) was taken (t=120 min). Following this, participants began cycling at 50% W_{max} for 60 min. Throughout the exercise, blood samples (5 ml) were taken at t=140, 150, 160 and 180 min (Fig. 1). The catheter was kept patent by flushing it with 3–4 ml isotonic saline (0.9%; Baxter, Norfolk, United Kingdom) after each blood sample. The experimental trial was then repeated 8 days later. The only difference in the trials on D0 and D8 was the consumption of a single bolus of GTE (600 mg catechins, 120 mg caffeine) or PLA after the collection of the resting blood sample and prior to the resting period.

2.1.4. Diet and supplement

After the initial trial, participants were randomly assigned to ingest either GTE or PLA for 7 days (Fig. 1). The GTE and PLA supplements were provided in the form of a drink. Each drink was provided in a 330-ml can. The GTE (Taiyo International, Japan) and PLA compositions are displayed in Table 1. Participants were instructed to consume two drinks per day, one drink an hour prior to breakfast and another drink an hour prior to dinner. The supplementation period started on the day following the presupplementation trial. Subjects received daily reminders in the form of text messages in the morning and the evening to ensure compliance. Participants returned to the human performance lab on D8 having consumed the drinks for 7 days. In the 24-h period prior to D0, trial participants were asked to record a food diary, which was replicated prior to D8 trial.

2.1.5. Sample collection

All blood samples were stored on ice for no longer than 35 min. Subsequently, plasma was separated by centrifugation (1500*g*, 10 min, 4°C), aliquoted in 1-ml samples and stored at −80°C.

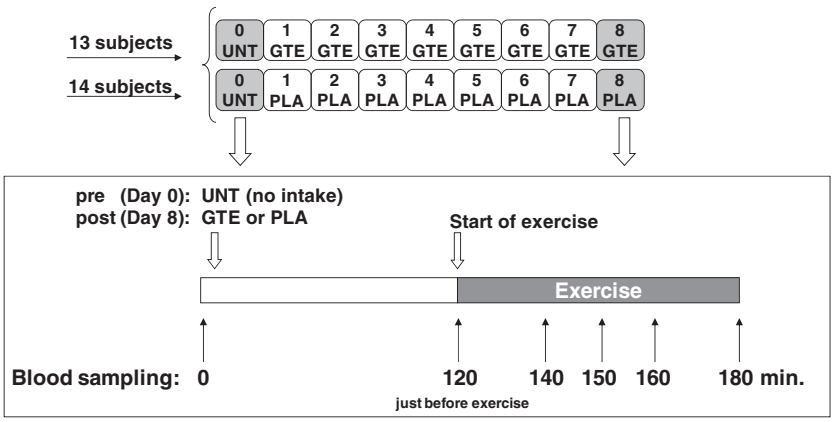

Fig. 1. Study design schematic.

Table 1
Content of the GTE and PLA drink

	Treatment	GTE	PLA
mg/330-ml can	Caffeine	120.4	10
	GC	55.1	0
	EGC	5.5	0
	C	181.1	0
	EC	47.0	0
	EGCG	207.5	0
	GCG	31.0	0
	ECG	25.6	0
	GC	0.0	0
Total mg/330-ml can	Catechins	559.0	0
	Caffeine	120.4	10
Total mg/day	Catechins	1119.0	0
	Caffeine	240.8	20

GC, gallocatechin; EGC, epigallocatechin; C, catechin; EC, epicatechin; GCG, gallocatechin gallate; ECG, epicatechin gallate.

2.2. Data acquisition

2.2.1. Metabolite profiling

Four-phase metabolite profiling and quantification of catecholamines (Fig. 2) were performed on human plasma samples at Metanomics Health GmbH, Berlin, Germany. Three types of mass spectrometry analyses were applied. GC–MS (Agilent 6890 GC coupled to an Agilent 5973 MS System, Agilent, Waldbronn, Germany) and LC–MS/MS [Agilent 1100 HPLC System (Agilent, Waldbronn, Germany) coupled to an Applied Biosystems API4000 MS/MS System (Applied Biosystems, Darmstadt, Germany)] were used for broad profiling, as described by van Ravenzwaay et al. [24]. Solid phase extraction (SPE)–LC–MS/MS [Symbiosis Pharma (Spark, Emmen, the Netherlands) coupled to an Applied Biosystems API4000 MS/MS System (Applied Biosystems, Darmstadt, Germany)] was used for the determination of catecholamine concentrations. A total of 228 metabolites fulfilled the quality criteria for relative quantification, and absolute quantification was performed for an additional 10 metabolites. From a total of 238 metabolites, 163 were known metabolites, and 75 were not chemically identified with sufficient certainty (i.e., thus considered in the present study to be unknown analytes).

Technical reference samples were measured in parallel with the study samples in order to allow the relative quantification of metabolites in the study samples. These technical reference samples were generated by pooling aliquots of plasma from all study samples. A relative quantification for each metabolite was obtained by normalizing peak intensity in the study samples to the median peak intensity of the corresponding metabolite in the technical reference samples measured in the same batch.

2.2.2. Metabolite profiling by GC–MS and LC–MS/MS

Proteins were removed from plasma samples (60 μl) by precipitation. Subsequently, polar and nonpolar plasma fractions were separated for both GC–MS and LC–MS/MS analyses by adding water and a mixture of ethanol and dichloromethane. For GC–MS analyses, the nonpolar fraction was treated with methanol under acidic conditions to yield the fatty acid methyl esters derived from both free fatty acids and hydrolyzed complex lipids. The polar and nonpolar fractions were further derivatized with *O*-methyl-hydroxyamine hydrochloride (20 mg/ml in pyridine, 50 μl) to convert oxo-groups to *O*-methyloximes and subsequently with a silylating agent (N-methyl-trimethylsilyltrifluoroacetamide (MSTFA), 50 μl) before GC–MS analysis [25]. For LC–MS/MS analyses, both fractions were reconstituted in appropriate solvent mixtures. High-performance LC was performed by gradient elution using methanol/water/formic acid on reversed-phase separation columns. Mass spectrometric detection technology was applied as described in the patent US 7196323, which allows targeted and high- sensitivity "Multiple Reaction Monitoring" profiling in parallel to a full screen analysis.

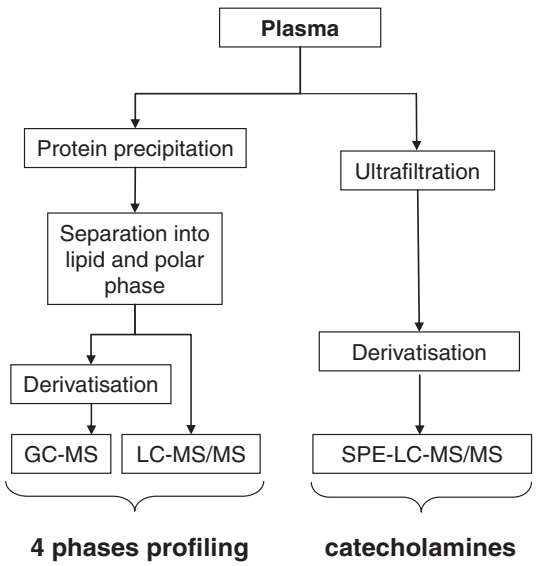

Fig. 2. Sample workflow: four-phase profiling and platform of catecholamines.

For the lipid phase, the broad profiling technology determines, e.g., fatty acid concentrations after acid/methanol treatment which is essential for derivatization preceding GC–MS analysis. As a consequence, complex lipids are hydrolyzed to components of the lipid backbone (i.e., glycerol) and fatty acids. Hence, the concentration of a fatty acid determined by this procedure represents the sum of its occurrence in free and in lipid-bound form. Components of the backbone can be recognized by the term ("lipid fraction") added to the metabolite name. As an example "glycerol, lipid fraction" represents glycerol liberated from complex lipids — in contrast, "glycerol, polar fraction" represents glycerol which had been present originally in the biological sample. The use of "additional" indicates that quantification can be affected by the co-occurrence of metabolites exhibiting identical characteristics in the analytical methods. Literature data and/or comparison with alternative methods (e.g., LC–MS/MS, GC–MS) suggests that such metabolites are present at minor concentrations only.

2.2.3. Quantification of catecholamines

Catecholamines and their related metabolites were measured by online SPE–LC–MS/MS, as described by Yamada et al. [26]. Quantification was performed using stable isotope-labeled standards.

2.3. Data analysis

The data set comprised profiles from 318 plasma samples that were analyzed using both multivariate and univariate statistical methods. (Six samples were not considered due to labeling errors.)

2.3.1. Multivariate analysis

Multivariate analysis including principal component analysis (PCA) and partial least squares/projection to latent structures-discriminant analysis (PLS-DA) was performed using SIMCA P+ version 12 software (Umetrics, Umea, Sweden). One subject was completely excluded from the analysis due to abnormal metabolite profiles. For another subject, one specific time point (D0, t=160 min) was discarded. All metabolite data were log-transformed to better match normal distribution. Data were further centered and scaled to unit variance. Scaling to unit variance introduces a common scale for all metabolites independent of their absolute amount of variance. Thereby, the resulting models obtain robustness, i.e., they cannot be dominated by a single or few high-variance metabolites. The explorative unsupervised multivariate analysis method PCA was used for the detection of trends, patterns and groupings among samples and variables. The supervised projection method PLS-DA was used to display the maximum covariance of metabolic data with a defined Y variable (class, categorical) in the data set. The cross-validated cumulative Q^2 value was used as a measure of the predictive value of the PLS-DA model. A Q^2 value of 1 indicates maximum predictive power, whereas Q^2 values close to or below 0 indicate a lack of predictive power. As a rule of thumb, models with Q^2_{cum}>50% are considered to be of good predictive power in this context. To account for the fact that several samples for each subject were analyzed, cross-validation was performed by the leave-one-subject out method. This ensured that cross-validation was not inappropriately facilitated by the presence of related samples (here: different time point from the same subject) in the test data set given the usually lower intraindividual variability compared to interindividual variability.

2.3.2. Univariate analysis

Analysis of variance (ANOVA) was performed using R-software package nlme [27,28]. Treatment factor "UNT" comprised all samples on D0, "GTE" all samples collected on D8 from subjects consuming GTE, and "PLA" all samples collected on D8 from subjects consuming placebo, respectively. The ANOVA mixed-effects model included the categorical fixed-effects factors "treatment" (UNT, PLA, GTE) and "time" (0, 120, 140, 150, 160, 180) and their interaction and random intercept effects for "day" and "subject." The combination of the binary factors "day" (0, 8) and the ternary factor "treatment" (UNT, GTE, PLA) allowed for setting up the following mixed-effects model without third-order interactions. The model specification (in R notation) was:

Fixed effects: metabolite ~ (treatment+time)^2,
Random effects: metabolite ~1|day/subject.

Table 2
ANOVA estimate for effect of exercise on metabolome derived from data from presupplementation (UNT)

METABOLITE \ time / min	140 vs 120	150 vs 120	160 vs 120	180 vs 120
Alanine	1.30	1.33	1.37	1.33
Cysteine (additional: Cystine)	1.07	1.06	1.10	1.11
Methionine	1.05	1.03	1.08	1.12
Phenylalanine	1.05	1.06	1.07	1.11
Tyrosine	1.04	1.05	1.07	1.13
4-Hydroxyphenylpyruvate	1.15	1.20	1.27	1.30
Ketoleucine	1.27	1.23	1.18	1.18
trans-4-Hydroxyproline	0.91	0.89	0.89	0.87
Urea	0.99	1.07	1.02	1.08
Erythrol	1.09	1.08	1.12	1.16
Glucosamine	1.16	1.12	1.12	1.18
Glucose	1.04	1.02	1.04	1.02
Mannosamine	1.14	1.12	1.17	1.17
Mannose	0.94	0.82	0.77	0.70
myo-Inositol	1.04	1.03	1.08	1.12
Sucrose	0.90	0.72	0.87	0.81
Cholestenol No 02	1.04	1.06	1.04	1.00
Cholesterol, total	1.06	1.07	1.05	1.04
Cholesterylester C16:0	1.10	0.97	1.01	1.00
Cholesterylester C18:1	1.02	1.10	1.10	1.09
Cholesterylester C18:2	1.03	1.09	1.07	1.12
Cholesterylester C20:4	1.02	1.04	1.02	1.08
Glycerol, lipid fraction	1.00	0.96	0.94	0.93
Pentadecanol	1.02	1.02	1.13	1.13
Taurochenodeoxycholic acid (additional: Taurodeoxycholic acid)	1.25	1.67	1.14	0.96
Arachidonic acid (C20:cis[5,8,11,14]4)	1.08	1.05	1.04	1.04
Behenic acid (C22:0)	1.08	1.04	1.05	1.02
conjugated Linoleic acid (C18:trans[9,11]2) (add.: conjugated Linoleic acid (C18:cis[9]trans[11]2))	1.06	1.07	1.08	1.07
dihomo-gamma-Linolenic acid	1.09	1.07	1.08	1.04
Docosahexaenoic acid (C22:cis[4,7,10,13,16,19]6)	1.09	1.05	1.04	1.05
Eicosapentaenoic acid (C20:cis[5,8,11,14,17]5)	1.08	1.04	1.01	1.01
gamma-Linolenic acid (C18:cis[6,9,12]3)	1.08	1.04	1.06	1.03
Isopalmitic acid (C16:0)	1.06	1.05	1.04	1.03
Lignoceric acid (C24:0)	1.05	1.01	1.03	1.03
Linoleic acid (C18:cis[9,12]2)	1.06	1.04	1.04	1.02
Myristic acid (C14:0)	1.05	1.06	1.09	1.17
Nervonic acid (C24:cis[15]1)	1.09	1.03	1.06	1.04
Palmitic acid (C16:0)	1.04	1.03	1.04	1.04
Palmitoleic acid (C16:cis[9]1)	1.06	1.06	1.07	1.10
Stearic acid (C18:0)	1.03	1.02	1.01	1.02
Tricosanoic acid (C23:0)	1.10	1.06	1.05	1.03
DAG (C18:1,C18:2)	1.09	1.09	1.10	1.12
TAG (C16:0,C16:1)	0.98	0.89	0.88	0.89
TAG (C16:0,C18:2)	0.97	0.91	0.93	0.93
TAG (C18:1,C18:2)	0.95	0.89	0.93	0.90
TAG (C18:2,C18:2)	0.98	0.97	0.97	0.93
Galactose, lipid fraction	1.09	1.04	1.07	1.05
Glucose, lipid fraction	1.20	1.15	1.26	1.10
myo-Inositol, lipid fraction	1.05	1.06	1.05	1.05
Cortisol	1.11	1.11	1.06	1.10
Dehydroepiandrosterone sulfate (additional:Testosterone-17-sulfate)	1.18	1.10	1.14	1.20
Pseudouridine	1.02	1.05	1.03	1.08
Uric acid	1.03	1.03	1.07	1.07
Uridine	1.04	1.04	1.09	1.10

METABOLITE \ time / min	140 vs 120	150 vs 120	160 vs 120	180 vs 120
Choline plasmalogen (C18,C20:4)	1.05	1.03	1.05	1.03
Glycerol phosphate, lipid fraction	1.11	1.08	1.06	1.01
Lysophosphatidylcholine (C18:0)	1.13	1.07	1.02	1.02
Lysophosphatidylcholine (C18:1)	0.98	0.95	0.99	0.90
Lysophosphatidylcholine (C18:2)	1.02	1.00	1.03	0.92
Lysophosphatidylcholine (C20:4)	0.99	0.97	0.94	0.91
Phosphatidylcholine (C16:0,C16:0)	1.04	1.04	1.02	1.03
Phosphatidylcholine (C16:1,C18:2)	1.01	1.03	1.06	1.03
Phosphatidylcholine (C18:0,C18:1)	1.02	1.02	1.01	1.00
Phosphatidylcholine (C18:0,C18:2)	0.99	1.00	0.99	1.00
Phosphatidylcholine (C18:0,C20:4)	1.00	1.00	1.01	1.01
Phosphatidylcholine (C18:0,C22:6)	1.04	1.03	1.02	1.03
Phosphatidylcholine (C18:2,C20:4) (additional: Phosphatidylcholine (C16:0,C22:6))	1.00	1.00	1.01	1.00
1-Hydroxy-2-amino-(cis,trans)-3,5-octadecadiene (from sphingolipids)	1.11	1.08	1.03	1.06
3-O-Methylsphingosine (add.: Sphingolipids, erythro-Sphingosine, threo-Sphingosine)	1.13	1.09	1.03	1.06
5-O-Methylsphingosine (add.: Sphingolipids, erythro-Sphingosine, threo-Sphingosine)	1.12	1.07	1.03	1.09
Ceramide (d18:1,C24:0)	1.11	1.07	1.05	1.05
Ceramide (d18:1,C24:1) (additional: Ceramide (d18:2,C24:0))	1.14	1.06	1.11	1.07
erythro-C16-Sphingosine	1.09	1.07	0.96	1.03
erythro-Dihydrosphingosine	1.10	1.06	1.02	1.05
erythro-Sphingosine (additional: Sphingolipids)	1.10	1.07	1.03	1.06
Sphingomyelin (d18:1,C16:0)	1.06	1.05	1.08	1.11
Sphingomyelin (d18:1,C24:0)	1.05	1.02	1.04	0.99
threo-Sphingosine (additional: Sphingolipids)	1.11	1.07	1.04	1.07
2-Hydroxybutyrate	0.81	0.75	0.72	0.74
3-Hydroxybutyrate	0.84	0.95	1.14	1.54
Citrate (additional: Isocitrate)	0.98	1.02	1.09	1.14
Fumarate	1.17	1.20	1.17	1.14
Lactate	**2.73**	**2.52**	**2.46**	**2.06**
Malate	1.85	1.89	1.84	1.79
Pyruvate (additional: Phosphoenolpyruvate (PEP))	**2.29**	**2.22**	**2.32**	**2.24**
Succinate	**2.08**	**2.16**	**2.33**	**2.32**
3,4-Dihydroxyphenylacetic acid (DOPAC)	1.10	1.21	1.24	1.40
3,4-Dihydroxyphenylalanine (DOPA)	1.05	1.11	1.12	1.14
3,4-Dihydroxyphenylglycol (DOPEG)	1.32	1.53	1.61	1.81
4-Hydroxy-3-methoxyphenylglycol (HMPG)	1.05	1.17	1.26	1.49
5-Hydroxy-3-indoleacetic acid (5-HIAA)	1.05	1.09	1.11	1.16
Homovanillic acid (HVA)	1.09	1.17	1.25	1.43
Noradrenaline (Norepinephrine)	**2.61**	**2.81**	**2.82**	**3.09**
Serotonin (5-HT)	1.16	0.96	1.20	1.49
Canthaxanthin	1.03	1.11	1.11	1.29
Creatine	1.08	1.12	1.17	1.26
Cryptoxanthin	1.13	1.07	1.09	1.06
Glycerol, polar fraction	1.44	1.62	1.79	**2.24**
Hippuric acid	1.17	1.21	1.30	1.28
Phosphocreatine	0.86	0.92	0.77	0.87
Taurine	1.04	1.04	1.13	1.20
4-Pyridoxic acid	1.11	1.11	1.18	1.20
alpha-Tocopherol	1.06	1.00	0.98	1.02
Coenzyme Q10	1.07	0.99	1.01	0.99
Nicotinamide	0.78	0.77	0.77	0.81
Pantothenic acid	1.31	1.27	1.35	1.37
Threonic acid	1.07	1.06	1.05	1.14

Data were transformed to ratio scale (10^x);
Color coding: ratio>1 & P value<.01 (■)
ratio>1 & .01≤P value<.05 (■)
ratio>1 & .05≤P value<.10 (■)
ratio<1 & .05≤P value<.10 (■)
ratio<1 & .01≤P value<.05 (■)
ratio<1 & P value<.01 (■)
Font coding: black & bold: (ratio<0.5) or (ratio>2)
black & normal: (0.5≤ratio<0.9) or (1.1<ratio≤2)
black & italic: 0.9≤ratio≤1.1.

The correlation between samples of the same subject at different times was handled by a model of type AR(p=1)=ARMA(p=1,q=0) with equidistant time steps time.int (1,2,…6). All study-relevant contrasts were read out in the form of treatment contrasts by selecting different factor reference levels. The t-statistics results of the ANOVA models comprised estimates, standard deviations, t values and P values.

Model diagnostics were conducted in four ways to ensure adequateness of model structure: (a) Residuals were inspected visually by scatter plots of standardized residuals versus fitted values: generally, residuals did not correlate with fitted values and showed homogenous variance. (b) Random subject intercepts were tested by t test comparing the two study arms GTE and PLA on D0. The number of significant metabolites was on false-positive level. (c) Random day effects were negligible relative to other variance components. Accordingly, they do not influence other effect estimates. (Note that the random day effect was introduced only due to technical/algorithmic reasons.) (d) The AR(1) correlation coefficient ("phi") was found to vary in a reasonable range with mean 0,14 and interquartile range from P25%=0.03 to P75%=0.24.

The t-statistics results of significantly changed metabolites are summarized in a color-coded format in Table 4. The resulting numbers of significantly changed metabolites were evaluated by binomial test to account for false positives due to multiple hypotheses testing (data not shown).

3. Results

The four-phase metabolite profiling using both GC–MS and LC–MS/MS (Fig. 2) comprised a total of 238 metabolites including 32 amino acids and amino acid derivatives; 11 carbohydrates and related metabolites; 10 metabolites related to energy metabolism; 3 nucleobases and related metabolites; 10 vitamin cofactors and related metabolites; 12 miscellaneous metabolites; 73 complex lipids, fatty acids and related metabolites; and 75 unknown analytes. In addition, 10 catecholamines and other monoamines were in most instances quantitatively measured using a targeted profiling method.

Initially, multivariate statistics (PCA and PLS-DA) was performed to identify outliers and to get a general idea on the variability of the data. PLS-DA clearly discriminated between exercise and resting conditions (Q^2_{cum}=80.5%). Furthermore, a clear separation was observed between pre- and post-GTE-supplementation (Q^2_{cum}=51.5% including all time points; Q^2_{cum}=49.2% including time points 140–180 min and subjects of the GTE study arm). Other models discriminating between the GTE and the PLA interventions under resting and exercise conditions, respectively, were not significant, indicating that interindividual differences might dominate the discrimination over treatment and/or exercise. Based on this overall impression, a univariate ANOVA mixed-effects model was calculated and clearly identified statistically significant changes in individual metabolites that could be attributed to the effect induced by exercise or the GTE supplement.

3.1. Effect of exercise on human metabolism

Table 2 illustrates the metabolite changes induced by moderate-intensity exercise in all subjects presupplementation (UNT). The changes during exercise (140, 150, 160, 180 min) were compared to resting conditions at the end of the 2-h rest period (120 min). They comprised of increases in indicators of glycolysis (lactate and pyruvate), indicators of lipolysis (glycerol), tricarboxylic acid cycle (TCA) cycle intermediates (citrate, fumarate, malate, succinate), indicators of adenine catabolism (uric acid), accelerated protein synthesis (uridine, pseudouridine), indicators of hormonal activity (cortisol, dehydroepiandrosterone sulfate), catecholamines [3,4 dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylglycol (DOPEG), 4-hydroxy-3-methoxyphenyl glycol (HMPG), 5-hydroxy-3-indoleacetic acid (5-HIAA), homovanillic acid (HVA), noradrenaline], vitamins and related metabolites (4-pyridoxic acid, pantothenic acid, cryptoxanthin), certain fatty acids (e.g., arachidonic acid, linoleic acid), certain phospholipids [e.g., choline plasmalogen, lysophosphatidylcholine (C18:0)], certain sphingolipids [e.g., ceramide (C18:1,C24:0), erythro-sphingosine], certain cholesteryl ester (e.g., cholesteryl ester C18:2), several amino acids (alanine, cysteine, methionine, phenylalanine, tyrosine) and miscellaneous metabolites (creatine, taurine, hippuric acid). Only a small number of these metabolites increased considerably during exercise with a ratio >2 (Table 2). These included lactate, pyruvate, succinate, noradrenaline and glycerol. On the other hand, some metabolites were reduced during exercise, such as 2-hydroxybutyrate, *trans*-4-hydroxyproline, mannose, certain triacylglycerides (TAGs) and nicotinamide. The onset of exercise caused a significant reduction in 3-hydroxybutyrate followed by a subsequent increase towards the end of exercise, indicating an initial increase in fat oxidation at the onset of exercise with a delay in fatty acid mobilization and availability late throughout exercise. The onset of exercise also increased the levels of certain fatty acids and sphingolipids. Other metabolites such as pseudouridine, uric acid, uridine, taurine, serotonin, and certain cholesteryl esters only showed changes towards the end of exercise.

Table 3
ANOVA estimate for effect of GTE on metabolome under fasting and resting condition

METABOLITE	PLA-D8 vs UNT-D0	GTE-D8 vs UNT-D0	GTE-D8 vs PLA-D8
Alanine	*1.10*	*0.93*	0.85
Asparagine	1.10	*1.01*	*0.91*
Threonine	*1.09*	*1.00*	*0.92*
Tryptophan	*1.04*	*0.93*	0.89
Ketoleucine	0.88	*0.97*	1.11
Urea	*1.00*	0.82	0.81
Cholesterylester C18:1	*0.93*	1.13	1.22
Cholesterylester C18:2	*0.91*	1.13	1.24
Cholesterylester C20:4	*0.95*	1.11	1.17
Glycerol, lipid fraction	1.15	0.87	0.76
Pentadecanol	*1.06*	0.88	0.84
Docosahexaenoic acid (C22:cis[4,7,10,13,16,19]6)	1.14	0.83	0.73
Eicosapentaenoic acid (C20:cis[5,8,11,14,17]5)	1.31	0.84	0.64
Isopalmitic acid (C16:0)	1.13	*0.92*	0.81
Myristic acid (C14:0)	1.23	*1.04*	0.85
DAG (C18:1,C18:2)	*1.06*	0.88	0.83
TAG (C16:0,C16:1)	1.27	0.87	0.69
TAG (C16:0,C18:2)	1.13	0.88	0.78
TAG (C18:2,C18:2)	*1.09*	0.82	0.75
TAG (C18:2,C18:3)	1.26	0.68	0.54
Phosphatidylcholine (C18:0,C22:6)	*1.05*	0.87	0.82
Phosphatidylcholine No 02	*1.06*	*0.94*	0.88
Sphingomyelin (d18:1,C16:0)	*0.91*	*1.07*	1.18
3-Hydroxybutyrate	0.74	1.63	**2.21**
Citrate (additional: Isocitrate)	*1.02*	1.22	1.20
Lactaldehyde	*0.93*	1.13	1.22
3,4-Dihydroxyphenylacetic acid (DOPAC)	1.20	1.61	1.34
5-Hydroxy-3-indoleacetic acid (5-HIAA)	*1.09*	*0.95*	0.87
Homovanillic acid (HVA)	*1.07*	1.50	1.41
Serotonin (5-HT)	1.39	0.73	0.52
Caffeine	1.45	**5.64**	**3.88**
Creatine	1.12	0.86	0.77
Glycerol, polar fraction	0.82	1.11	1.36
Hippuric acid	0.80	1.64	**2.04**
Ascorbic acid (additional: Glucose)	1.13	*1.03*	*0.91*

Data were transformed to ratio scale (10^x);
Color coding: ratio>1 & P value<.01 (■)
ratio>1 & .01≤P value<.05 (■)
ratio>1 & .05≤P value<.10 (■)
ratio<1 & .05≤P value<.10 (■)
ratio<1 & .01≤P value<.05 (■)
ratio<1 & P value<.01 (■)
Font coding: black & bold: (ratio<0.5) or (ratio>2)
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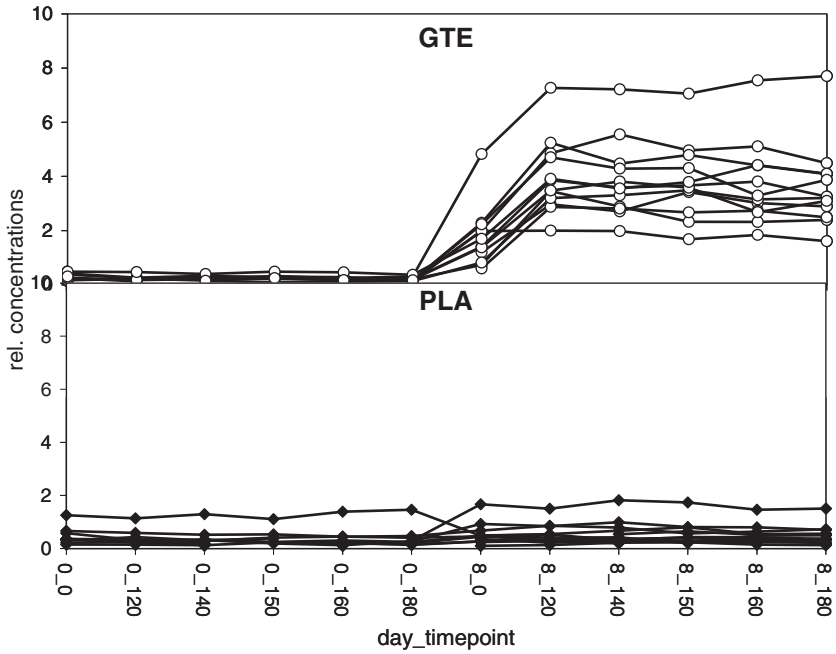

Fig. 3. Relative concentrations of caffeine.

3.2. Effect of GTE under resting condition

The effect of GTE under resting conditions was assessed by comparing the metabolite profiles from post-GTE-supplementation (GTE-D8) versus presupplementation (UNT-D0) and from post-GTE-supplementation (GTE-D8) versus post-PLA-supplementation (PLA-D8). As a control, the profiles from post-PLA-supplementation (PLA-D8) were compared to presupplementation (UNT-D0) (Table 3). To assess the impact of 7-day supplementation of GTE under resting fasted conditions, only the profiles recorded at time point 0 on each intervention day were taken into account.

Caffeine, hippuric acid, homovanillic acid and 3,4-dihydroxyphenylacetate were elevated post-GTE-supplementation. Caffeine was present in the GTE supplement, and hence, the presence of caffeine in plasma on D8 was considered as a measure of compliance. The relative concentrations of caffeine are displayed in Fig. 3. hippuric acid, homovanillic acid and 3,4-dihydroxyphenylacetate are known metabolites from gut microbial degradation of polyphenols [29]. Besides these exogenous effects, GTE induced reductions in diacylglyceride (DAG) and TAG, certain fatty acids, certain phosphatidylcholines, alanine, tryptophan, creatine, urea, serotonin and 5-HIAA. Higher concentrations were observed for certain cholesteryl esters, 3-hydroxybutyrate, citrate, lactaldehyde and glycerol (Fig. 4).

3.3. Effect of GTE during exercise

The ANOVA mixed-effects model segregated the effects induced by GTE or PLA during exercise from those induced by exercise alone. Table 4 lists the metabolite changes solely caused by GTE or PLA during exercise. The following comparisons were made: post-GTE-supplementation (GTE-D8) versus presupplementation (UNT-D0), post-GTE-supplementation (GTE-D8) versus post-PLA-supplementation (PLA-D8) and — as a control — post-PLA-supplementation (PLA-D8) versus presupplementation (UNT-D0).

The effects of GTE or PLA during exercise were assessed after 20, 30, 40 and 60 min of exercise. The data were normalized to the end of the 2-h rest period (t=120 min) (Fig. 1) to focus on the effects induced during exercise and to eliminate any effects that accounted for resting conditions and were apparent in shifts in baseline levels at time points 0 and 120 min. Our study design included the 7-day (chronic) supplementation as well as the single bolus (acute) intake of either GTE or PLA on D8. It is thought that 7-day GTE supplementation would mostly affect plasma metabolites that were measured under resting conditions at time point 0, whereas the single bolus intake prior to the trial on D8 would determine the effects during exercise. The reason for this is that the main components of GTE would have accumulated in plasma after the 2 h of rest [29]. Thus, by normalizing the metabolite changes to the end of the 2-h rest period (120 min), the effects during exercise may at first instance be ascribed to the main components in GTE boosted by the single bolus intake.

Overall, the metabolite changes induced by GTE during exercise were weaker than the effects induced by exercise itself. Nevertheless, significant metabolite changes were observed when comparing both the effect of GTE-D8 versus UNT-D0 and the effect of GTE-D8 versus PLA-D8 (Table 4). These changes comprised of the increase in alanine, cysteine, histidine, certain cholesteryl esters, lactate, malate, pyruvate, hippurate and phosphocreatine. In addition, reductions in 3-hydroxybutyrate, certain fatty acids, certain TAGs and DOPEG were apparent during exercise (Fig. 4). Other changes could not fully be attributed to the GTE supplementation. These may be due to day-to-day variations or the PLA supplementation. For example, glycerol, glutamate, TAG (C16:0,C16:1), noradrenaline, serotonin and taurine were reduced in GTE-D8 when compared to PLA-D8. However, these changes were not seen when comparing GTE-D8 versus UNT-D0. On the other hand, these changes were increased when comparing the effect of PLA-D8 versus UNT-D0.

4. Discussion

By applying untargeted GC–MS- and LC–MS-based metabolite profiling to human plasma, we have been able to comprehensively investigate the metabolic responses following GTE supplementation at rest and during moderate-intensity exercise. The main finding of the current study was that 7-day GTE supplementation resulted in different metabolic effects during rest and exercise when compared

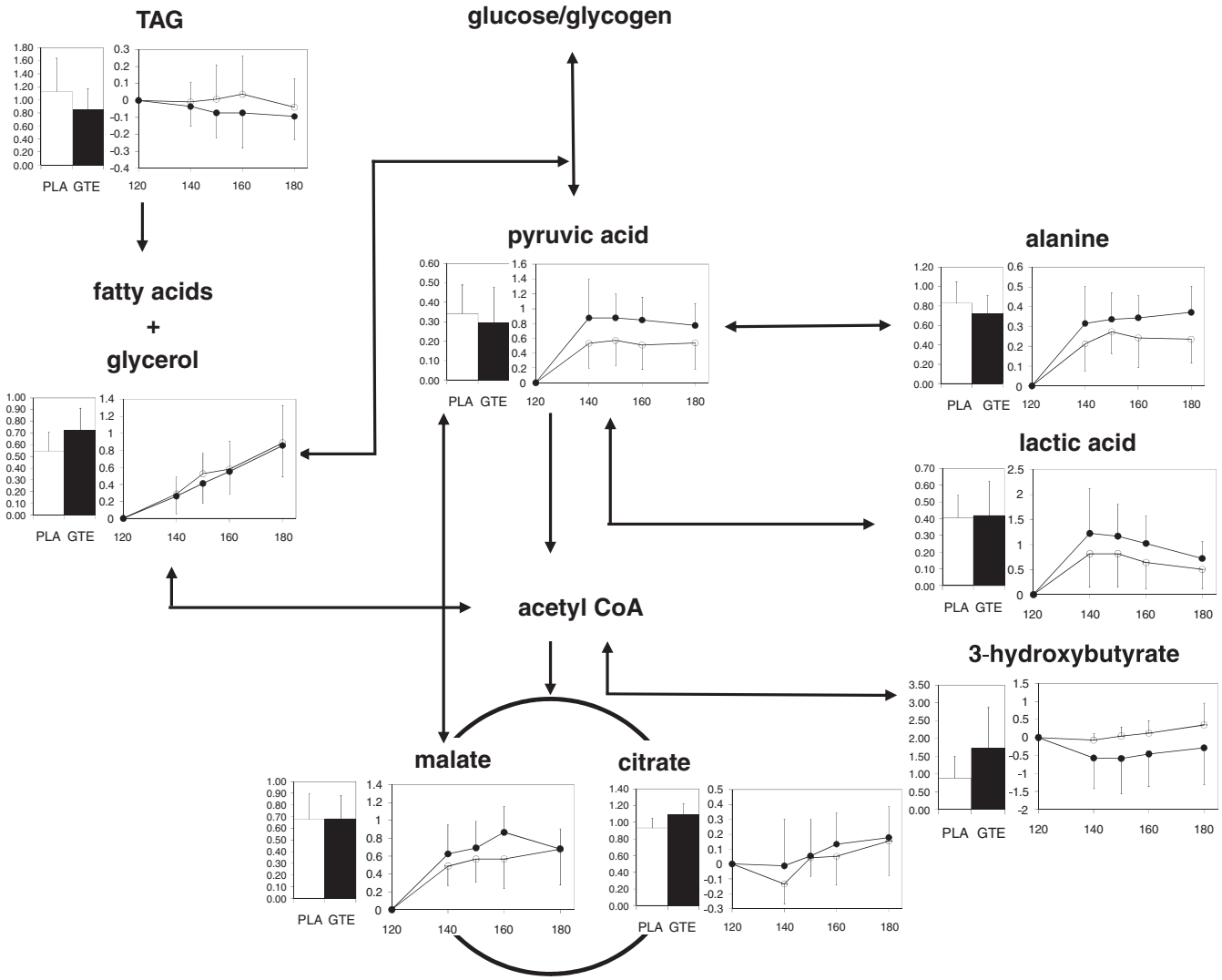

Fig. 4. Plasma metabolite concentrations of GTE versus PLA at rest (bar graph) and during exercise (line graph) illustrated as part of the intermediary metabolism. Black circles and white circles represent GTE and PLA, respectively. Data are means±S.D. The *x*-axis of the line graphs indicates time in minutes.

to PLA and UNT. In particular, GTE altered metabolite concentrations involved in the intermediary metabolism (Fig. 4). At rest, GTE induced metabolite changes indicative of enhanced lipolysis (increase in glycerol, reduction in TAGs, increased fat oxidation, increase in 3-hydroxybutyrate), activated TCA cycle (increase in citrate), reduced amino acid catabolism (reduction in urea and creatine), reduced Cori cycle (reduction in alanine) and enhanced glycolysis (increase in lactaldehyde). These metabolite changes suggest that GTE has the potential to enhance aerobic energy and fat metabolism at rest. Metabolic effects related to oxidative energy metabolism have previously been observed using a ^{1}H NMR metabolic profiling in human urine following 2 days of GTE supplementation [30]. Our results are also in line with a number of studies that have demonstrated increases in whole-body EE and fat oxidation during resting conditions following GTE supplementation [7,11,12]. However, a number of studies have reported no difference in FFA or glycerol concentrations despite changes in whole-body measures of fat oxidation [13,31,32]. In the current study, fat oxidation rates were not measured during the rest period. Therefore, it cannot finally be concluded whether the metabolite changes related to fat metabolism (glycerol or 3-hydroxybutyrate) would have induced changes in fat oxidation.

In comparison, GTE supplementation resulted in contrasting effects during exercise. GTE caused metabolite changes that are associated with enhanced glycolysis (increase in pyruvate, lactate), enhanced Cori cycle (increase in alanine) and decreased fat oxidation (reduction in 3-hydroxybutyrate) when compared to PLA and UNT. These changes suggest an enhancement in anaerobic energy metabolism. This result is somewhat unexpected since several previous studies have reported that acute [15] and chronic [16] GTE supplementation has the ability to induce greater fat oxidation rates over and above those of exercise alone, potentially through stimulating lipolysis as illustrated by higher concentrations of glycerol [15]. In the current study, no change in lipolytic metabolites (glycerol) was observed during exercise. This may be explained in part by the increase in lactate concentration considering that lactate is known to inhibit lipolysis and limit fat oxidation rates during exercise [33]. The high lactate concentrations were unexpected due to the moderate exercise intensity implemented in the study. The accumulation of pyruvate and lactate and the reduction in 3-hydroxybutyrate indicate a lower pyruvate oxidation through acetyl-CoA (Fig. 4). It is notable that we observed no difference in fat oxidation rates during exercise following GTE supplementation when compared to PLA and UNT (data not shown), suggesting that

Table 4
ANOVA estimate for effect of GTE on metabolome during exercise

	PLA-D8 vs UNT-D0				GTE-D8 vs UNT-D0				GTE-D8 vs PLA-D8			
time / min METABOLITE	140 vs 120	150 vs 120	160 vs 120	180 vs 120	140 vs 120	150 vs 120	160 vs 120	180 vs 120	140 vs 120	150 vs 120	160 vs 120	180 vs 120
Alanine	*0.97*	*1.00*	*0.94*	*0.97*	1.12	1.14	1.11	1.18	1.16	1.14	1.19	1.22
Aspartate	1.15	1.18	1.26	1.36	1.17	1.13	1.44	1.22	*1.02*	*0.96*	1.14	0.90
Cysteine (additional: Cystine)	*1.00*	*1.00*	*0.95*	*0.94*	1.16	1.13	*1.07*	1.10	1.16	1.14	1.13	1.17
Glutamate	1.13	1.26	*1.08*	1.21	*0.94*	*1.02*	0.85	*0.93*	0.83	0.81	0.79	0.77
Histidine	*0.95*	*0.99*	*0.98*	*0.97*	*1.08*	*1.07*	*1.08*	*1.09*	1.14	*1.09*	1.10	1.12
Urea	*0.97*	0.86	0.89	0.86	*1.02*	0.88	*0.95*	0.88	*1.05*	*1.03*	*1.07*	*1.02*
Sucrose	1.71	1.36	1.48	1.75	1.51	1.75	1.24	1.12	0.88	1.29	0.84	0.64
Cholesterylester C18:2	*1.00*	*1.04*	*1.00*	*0.93*	*1.06*	*1.04*	*1.06*	*1.05*	*1.05*	*1.00*	*1.07*	1.13
Cholesterylester C20:4	*0.96*	*0.97*	*0.98*	*0.95*	*1.00*	*1.00*	*1.06*	*1.03*	*1.03*	*1.03*	*1.08*	*1.09*
conjugated Linoleic acid (C18:trans[9,11]2) (add.: conjugated Linoleic acid (C18:cis[9]trans[11]2))	*0.93*	*0.97*	*0.98*	*0.95*	*0.91*	*0.93*	*0.91*	*0.96*	*0.98*	*0.96*	*0.93*	*1.01*
dihomo-gamma-Linolenic acid (C20:cis[8,11,14]3)	*0.93*	*0.97*	*0.96*	*0.98*	*0.95*	*0.98*	*0.94*	*0.99*	*1.03*	*1.01*	*0.99*	*1.01*
Eicosanoic acid (C20:0)	*1.02*	*1.09*	*1.07*	*1.09*	*1.01*	*1.06*	*0.98*	*1.08*	*0.99*	*0.97*	*0.92*	*0.99*
gamma-Linolenic acid (C18:cis[6,9,12]3)	0.89	*0.91*	*0.92*	0.88	*0.94*	*0.98*	*0.91*	*0.98*	*1.05*	*1.09*	*0.99*	1.11
Heptadecanoic acid (C17:0)	*0.98*	*1.08*	*1.10*	*1.03*	*0.96*	*0.97*	*0.99*	*0.92*	*0.99*	0.90	0.90	0.89
Linoleic acid (C18:cis[9,12]2)	*0.98*	*0.98*	*1.01*	*1.01*	*0.95*	*0.95*	*0.93*	*0.99*	*0.97*	*0.97*	*0.92*	*0.98*
Myristic acid (C14:0)	*0.95*	*0.99*	*1.02*	*0.93*	*0.89*	*0.95*	*0.91*	*0.93*	*0.94*	*0.96*	0.89	*1.00*
Oleic acid (C18:cis[9]1)	*0.99*	*1.01*	*1.04*	*1.02*	*0.92*	*0.93*	*0.92*	*0.98*	*0.93*	*0.93*	0.89	*0.97*
Palmitic acid (C16:0)	*0.98*	*0.99*	*1.02*	*1.01*	*0.94*	*0.96*	*0.93*	*0.99*	*0.96*	*0.97*	*0.91*	*0.98*
Palmitoleic acid (C16:cis[9]1)	*0.99*	*1.02*	*1.06*	*1.04*	0.87	*0.91*	0.88	*0.95*	0.88	0.89	0.84	*0.91*
TAG (C16:0,C16:1)	*1.09*	1.19	1.14	1.15	*0.98*	*1.02*	*0.96*	*0.97*	*0.90*	0.86	0.84	0.85
TAG (C16:0,C18:2)	*1.09*	*1.09*	*1.06*	*1.06*	*0.99*	*0.97*	*0.96*	*0.94*	*0.91*	0.90	0.90	0.89
TAG (C18:2,C18:2)	*1.01*	*1.04*	*1.07*	*1.02*	*0.96*	*0.91*	*0.92*	*0.94*	*0.95*	0.88	0.86	*0.91*
TAG (C18:2,C18:3)	1.11	1.12	*0.96*	*1.07*	*0.91*	*0.93*	0.79	*0.92*	0.82	0.83	0.82	0.86
myo-Inositol, lipid fraction	*0.96*	*0.96*	*0.95*	*0.94*	*0.94*	*0.95*	*0.91*	*0.92*	*0.99*	*0.99*	*0.96*	*0.98*
Choline plasmalogen (C18,C20:4)	*1.02*	*1.03*	*0.99*	*1.01*	*0.95*	*0.98*	*0.95*	*0.99*	*0.94*	*0.95*	*0.96*	*0.98*
Lysophosphatidylcholine (C18:2)	*0.98*	*0.95*	0.87	*0.95*	*0.96*	*0.91*	0.87	0.88	*0.98*	*0.96*	*1.00*	*0.93*
Phosphatidylcholine (C16:0,C20:4) (additional: Phosphatidylcholine (C18:2,C18:2))	*0.99*	*1.00*	*0.99*	*0.99*	*1.00*	*1.01*	*1.01*	*1.01*	*1.01*	*1.01*	*1.02*	*1.02*
Phosphatidylcholine (C18:0,C20:4)	*0.99*	*1.00*	*0.99*	*0.99*	*1.01*	*1.02*	*0.98*	*1.03*	*1.02*	*1.03*	*1.00*	*1.04*
Phosphatidylcholine (C18:0,C22:6)	*1.00*	*1.02*	*1.00*	*0.99*	*0.98*	*0.96*	*0.99*	*0.97*	*0.98*	*0.95*	*0.99*	*0.98*
Phosphatidylcholine (C18:2,C20:4) (additional: Phosphatidylcholine (C16:0,C22:6))	*1.01*	*1.02*	*1.01*	*1.01*	*0.99*	*1.00*	*0.99*	*1.00*	*0.97*	*0.98*	*0.99*	*1.00*
Phosphatidylcholine No 02	*1.02*	*0.99*	*1.01*	*1.05*	1.10	*1.05*	1.13	*1.10*	*1.08*	*1.05*	1.11	*1.05*
Ceramide (d18:1,C24:1) (additional: Ceramide (d18:2,C24:0))	*1.04*	1.14	*1.02*	*1.06*	*0.91*	*0.99*	*0.95*	*1.01*	0.88	0.87	*0.93*	*0.95*
Sphingomyelin (d18:1,C16:0)	*1.00*	*0.98*	*0.98*	0.83	*0.92*	*0.95*	*0.98*	*0.99*	*0.92*	*0.96*	*1.00*	1.19
3-Hydroxybutyrate	*1.08*	1.18	1.11	*1.06*	0.77	0.73	0.73	0.69	0.71	0.62	0.66	0.65
Lactaldehyde	*1.09*	*1.03*	*1.06*	1.14	*1.04*	0.87	*1.03*	1.24	*0.96*	0.85	*0.97*	*1.09*
Lactate	*1.03*	1.11	*1.03*	1.10	1.38	1.54	1.45	1.39	1.34	1.39	1.41	1.26
Malate	*1.02*	*1.07*	1.11	1.21	1.15	1.21	1.40	1.26	1.12	1.12	1.26	*1.04*
Pyruvate (add.: Phosphoenolpyruvate (PEP))	*1.03*	*1.10*	*1.00*	*1.07*	1.31	1.42	1.34	1.32	1.27	1.30	1.34	1.24
Succinate	*0.93*	*1.03*	*0.90*	*0.99*	1.15	1.12	*1.07*	*1.03*	1.23	*1.09*	1.18	*1.03*
3,4-Dihydroxyphenylglycol (DOPEG)	*1.08*	*1.02*	*1.09*	*1.05*	*0.94*	*0.92*	0.88	0.90	0.87	0.90	0.81	0.86
Noradrenaline (Norepinephrine)	*1.09*	1.16	1.27	1.31	*1.02*	*1.06*	1.12	*1.00*	*0.93*	*0.92*	0.88	0.77
Serotonin (5-HT)	1.11	**2.12**	1.42	**2.86**	0.75	0.86	0.88	0.83	0.67	**0.41**	0.62	**0.29**
Cortisol	1.16	1.22	1.14	*1.08*	*0.94*	*0.94*	*0.96*	*1.03*	0.81	0.77	0.84	*0.96*
Dehydroepiandrosterone sulfate (additional: Testosterone-17-sulfate)	*0.99*	*1.04*	*1.05*	*1.05*	0.85	0.87	0.81	*0.97*	0.85	0.84	0.78	*0.93*
Canthaxanthin	*1.08*	1.22	*1.09*	1.20	*0.95*	0.90	0.87	0.88	0.88	0.73	0.80	0.73
Glycerol, polar fraction	1.18	1.27	1.21	1.24	*0.96*	*0.99*	*1.00*	*1.03*	0.81	0.78	0.83	0.83
Hippuric acid	*1.03*	1.24	*1.06*	*1.00*	1.27	1.37	1.32	1.57	1.23	1.10	1.24	1.57
Phosphocreatine	0.85	0.71	1.17	*0.93*	1.43	1.24	1.51	1.34	1.68	1.75	1.29	1.44
Taurine	*1.07*	1.13	*1.06*	1.22	*0.96*	*0.98*	*0.95*	*0.99*	0.90	0.87	0.90	0.81
4-Pyridoxic acid	0.86	0.90	0.81	0.82	*1.05*	*1.10*	*0.98*	*1.00*	1.22	1.22	1.21	1.21
beta-Carotene	*1.03*	*1.10*	*1.03*	*0.98*	0.89	*1.06*	*1.07*	1.14	0.86	*0.96*	*1.04*	1.17

Data were transformed to ratio scale (10^x);
Color coding:ratio>1 & P value<.01 (■)
ratio>1 & .01≤P value<.05 (■)
ratio>1 & .05≤P value<.10 (■)
ratio<1 & .05≤P value<.10 (■)
ratio<1 & .01≤P value<.05 (■)
ratio<1 & P value<.01 (■)
Font coding: black & bold: (ratio<0.5) or (ratio>2)
black & normal: (0.5≤ratio<0.9) or (1.1<ratio≤2)
black & italic: 0.9≤ratio≤1.1.

these metabolite changes were too weak to cause changes in physiological parameters.

In the current study, we also used targeted profiling of catecholamines in order to elucidate whether GTE supplementation is able to stimulate the adrenergic system. Fat metabolism can be activated by stimulating the adrenergic response. Not surprisingly, in the present study, exercise alone induced significant changes of 14 metabolites involved in the catecholamine metabolism. EGCG, a major catechin in GTE, has been suggested to directly inhibit COMT [18], an enzyme that is responsible for degrading catecholamines (NA and A). The current hypothesis is that degrading these catecholamines leads to attenuation of sympathetic nervous system activity. The inhibition of COMT and thus reduced degradation of NA would result in an increase in EE, lipolysis and potentially an increase in fat oxidation [10]. A limited number of studies have shown that GTE supplementation increases catecholamine concentrations following acute GTE supplementation [14,34,35]. However, the actual contribution of this mechanism to reported effects of GTE in humans remains unclear. In our study, GTE did not increase NA and directly related metabolites either at rest or during exercise. This result suggests that GTE supplementation may not alter the activity of COMT and thus questions the efficacy of the widely referenced COMT mechanism in humans. Interestingly, during the resting condition, metabolites indicative of lipolysis were elevated, which may occur through a cascade of events activated by catecholamines of the adrenergic system [33]. However, since no differences in NA and A were observed also during resting conditions, this could suggest that GTE stimulated lipolysis via nonadrenergic mechanisms. The nonadrenergic mechanisms could include the up-regulation of lipid-metabolizing enzymes by nuclear factor-κB [36]. Alternatively, augmented lipolysis may be explained by the action of caffeine rather than the effects of green tea catechins. Caffeine has been shown to stimulate lipolysis independent of changes in catecholamines [37] via antagonizing adenosine receptors directly on adipocytes [38]. However, it is not known whether green tea catechins are able to elicit direct effects on adipose tissue similar to those of caffeine.

This is the first study to directly compare the metabolic responses to GTE supplementation between resting and during moderate-intensity exercise conditions. Significant differences in metabolic responses at rest and during exercise following GTE supplementation were observed. Similar results were observed in a previous study showing that the ingestion of capsinoids — which are nonpungent analogues of capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) naturally present in sweet pepper — increased adrenergic activity and energy expenditure at rest but had little effect during exercise [39]. It could be argued that, during exercise, GTE was not potent enough to further enhance an already stimulated environment. In comparison, GTE may offer stimulatory effects to the human metabolism under resting conditions. The apparent difference in the metabolic response at rest and during exercise may be rooted in differences between chronic and acute GTE supplementation. In the current study, the metabolic effects observed during exercise can largely be attributed to the acute (single dose) post-GTE-supplementation, while the effects at rest account for the chronic (7 days) GTE supplementation. Thus, an increase in EE and fat oxidation and their related metabolic effects may only be present after chronic GTE supplementation. Chronic supplementation may induce a progressive regulation of various proteins and enzymes at certain sites in the body resulting in a systemic shift on the endogenous metabolism after long-term supplementation. For example, chronic GTE supplementation (8–24 weeks) has been demonstrated to alter the gene expression related to energy metabolism and lipid homeostasis in an animal model [40].

Alternatively, the apparent difference in the metabolic effects at rest and during exercise may be attributed to different bioactive components in GTE and/or the presence of caffeine, known to be crucial factors ultimately influencing the rates of fat or carbohydrate oxidation [41]. The single dose of GTE or PLA consumed 2 h before the exercise test during the postsupplementation trial increased the concentrations of directly absorbed components in GTE such as catechins, in particular EGCG, and caffeine (Fig. 3) and may be responsible for the effects observed during exercise. In comparison, phenolic metabolites originating from gut microbial degradation of polyphenols usually only appear in circulation 3–4 h after polyphenol consumption [29] and are unlikely to be responsible for the effect during exercise. However, they could have contributed to the chronic effects observed at rest. Following 7-day GTE supplementation, some phenolic acids such as hippuric acid, homovanillic acid and 3,4-dihydroxyphenylacetic acid were increased in plasma at rest due to the slow bioconversion of polyphenols by gut microbiota. Nutrient–gut microbiota interactions have previously been discussed to play an important role in polyphenol bioavailability, human obesity and insulin resistance [41]. Further investigations are needed to assign acute and chronic effects to certain bioactive species in GTE and to specify the role of gut microbiota.

The current study also illustrated novel metabolic responses of GTE at rest and during exercise. GTE induced reductions in metabolites from the tryptophan pathway (tryptophan, serotonin and 5-HIAA) at rest and in DOPEG, serotonin and NA during exercise. DOPEG is known to inhibit the enzyme monoamine oxidase, causing the oxidative deamination of NA, serotonin and other amines. Inhibition of this enzyme would lead to increasing concentrations of NA and serotonin. Yet these two metabolites were reduced at the end of the exercise period, suggesting a complex relationship between DOPEG, NA and serotonin as observed in plasma. These data provide evidence that GTE is capable of altering activity of certain catecholamines. However, the underlying mechanisms as well as their relation to physiological outcomes remain to be elucidated.

Finally, changes in certain TAGs and fatty acids were observed post-GTE-supplementation under resting and exercise conditions. This may suggest that GTE affects the response of exogenous and endogenous lipids. Manipulating lipids could be an interesting target for protecting against insulin resistance. Elevation in circulating lipids (DAG, TAG, FFA) has commonly been associated with impaired insulin-mediated glucose uptake [42,43] and elevated insulin resistance [44] usually coexisting with obesity and type 2 diabetes [45]. Exercise is one way of providing protection by having beneficial effects on glycemic control [46] and intramuscular lipids [47]. Recent evidence has shown that chronic GTE supplementation suppresses insulin resistance mainly in animal models [39,48] and improves insulin sensitivity in young physically active males [15]. However, the protective effects against insulin resistance with GTE in humans are currently not conclusive [49]. It is interesting to consider the possibility that GTE may provide additive protection against insulin resistance when combined with exercise by attenuating intramuscular lipids.

In summary, our comprehensive metabolite profiling approach broadened our understanding on the mode of action of exercise and GTE beyond the physiological outcomes. Moderate-intensity exercise stimulated multiple metabolic pathways including lipolysis, glycolysis, as well as the activation of the TCA cycle and the adrenergic system. The metabolite changes induced by GTE were more subtle and affected fewer pathways when compared to those induced by exercise alone. In agreement with previous studies, we illustrated that 7-day GTE supplementation mainly enhanced metabolites indicative of lipolysis and fat oxidation under resting conditions when compared to PLA. This effect was not enhanced during exercise. This suggests that a single dose of GTE may not be potent enough to stimulate further the metabolism that was already up-regulated by exercise. Furthermore, GTE did not stimulate the adrenergic system during rest and exercise since no increase in NA and related catecholamines was observed. This challenges COMT inhibition as

the putative mechanism of action of GTE *in vivo*. Yet GTE stimulated lipolysis under resting conditions, suggesting nonadrenergic mechanisms. It is clear that the effects observed in plasma only provide limited information on the underlying mechanisms of action since the metabolite concentrations in plasma reflect spillover effects that may occur in several tissues including the brain, skeletal muscle, liver, gut and adipose tissue. Further investigations are required to elucidate the potential targets, interactions and bioactivity of green tea catechins to understand the exact mechanisms of GTE and the physiological outcomes in the human body.

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